Transgenic Animal Expressing Alzheimer's Tau Protein

Neurodegenerative diseases represent a heterogeneous group of genetic and acquired neurological disorders that result in severe and progressive cognitive and motor impairment with on-set during mid- to late-life (1). The most common cause of dementia is Alzheimer's disease. In less than 5% of the cases Alzheimer's disease genetic factors are involved, the rest of cases are sporadic.

Tau protein is the protein expressed in central nervous system and plays a critical role in the neuronal architecture by sta-bilizing intracellular microtubule network. Impairment of the physiological role of the tau protein either by truncation, hy-perphosphorylation or by disturbing the balance between the six naturally occurring tau isoforms leads to the formation of neu-rofibrillary tangles (NFT), dystrophic neurites and neuropil threads. These structures represent ultrastructural hallmarks of Alzheimer's disease. The major protein subunit of these struc-tures is microtubule associated protein tau (2,3). The amount of NFT found in autopsies of AD patients correlates with clinic-al symptoms including intellectual decline. Therefore tau pro-tein plays a critical role in AD pathology. The recent discovery of cosegregation of specific mutations in the tau gene with the disease frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) has confirmed that certain abnormalities in the tau protein can be a primary cause of neurodegeneration and dementia in affected individuals (4,5). However there is no experimental model that would directly address the details on the role of tau protein in pathogenesis of AD.

Recent transgenic models of AD are based on expression of pro-teins associated with FAD (APP, PS1, ApoE etc.) either mutated or not. They include single and multigenic humanized animals. However none of the models recapitulates both hallmarks of AD, they usually do not show neurofibrillary pathology and none of them includes the possibility of investigation the function of environmental risk factors, which play a major role in the de-velopment of AD.

Currently available animal models showing some neurofibrillary
changes are based solely on transgenic expression of mutated tau
derived from frontotemporal dementia linked to chromosome 17
(FTDP17). Typical example of this group is JNPL3 transgenic
mouse (6). Another evidence on the role of mutated tau have been
published recently by Goedert and Spillantini (7).

In another transgenic animal model an overexpression of four re-
peat tau protein was investigated. Prominent axonopathy in the
brain and spinal cord was reported and it was shown that an
overexpression of four repeat tau protein isoform is sufficient
to alter physiology of neurons. In the model, axonal dilations
with accumulation of neurofilaments were documented in the neur-
ons of central nervous system however without formation, however
of intraneuronal neurofibrillary tangles (8).

The transgenic mouse was also produced in order to examine the
normal cellular function of tau and its role in pathogenesis.
The transgenic mouse overexpress a genomic tau transgene and
also tau cDNA transgene. The comparison of two models shows that
the distribution of tau is similar in both transgenic lines.
Tau-immunoreactive axonal swellings were found in the spinal
cords of the cDNA mice, which correlated with a hind-limb abnor-
mality, whereas neuropathology was not observed in the genomic
mice (9).

Despite of the advantage of rat as an experimental model in
neurobiology in comparison to mice, it is surprising that no tau
transgenic model for neurofibrillary pathology was produced.
In general it is considerable lower amount of transgenic rat
models of neurodegeneration produced till now. None of them re-
capitulates tau pathology, which is typical for AD.

Czech C, et al. (12) described a transgenic rat model which com-
prises of mutations in the gene coded for presenilin 1 (SP1).
PS1 is considered as a cause for the majority of cases of early
onset familial Alzheimer's disease, however the published model
did not show sufficient signs of AD associated pathology.
Echeverria V and Cuello AC. (13) comment on the role of intra-

cellular accumulation of beta amyloid and the possible neuro-
pathological role of intracellular amyloid-beta accumulation in
Alzheimer's disease. Their present recent observations from a
rat transgenic model with a phenotype of intracellular accumula-
tion of A-beta fragments in neurons of the hippocampus and cor-
tex, however without plaque and NFT formation. Therefore for
rats, which is the second most often used animal in transgenic
studies applies the same as for mice: there is no transgenic rat
described in scientific literature that would express truncated
tau protein and showed NFT formation.

Till now there is no experimental model available for investiga-
tion of etiology of AD in a connection with hypertension or oth-
er risk factors like diabetes and dislipidemia, which are asso-
ciated with AD. Genetic background of experimental transgenic
animals plays a role in the development of appropriate transgen-
ic model

It is well known that AD is associated with certain environment-
al risk factors. It was shown in epidemiological studies, that
risk factors for vascular disease and stroke are associated with
cognitive impairment and Alzheimer's disease; moreover the pres-
ence of cerebrovascular disease intensifies the presence and
severity of the clinical symptoms of Alzheimer's disease (14).
Another link between hypertension and dementia of AD type is in-
dicated in a Rotterdam Study (15).

Therefore it is important to develop an experimental model for
AD, which would mimic the main pathological features of the dis-
ease and simultaneously allows an expression of environmental
factors associated with AD etiology.

It is therefore an object of present invention to provide an ex-
perimental model, which reflects the hallmarks of AD as close as
possible and also the assay system for substances for treatment,
prevention and diagnosis of AD.

The present invention relates to a transgenic non-human animal
expressing cDNA encoded Alzheimer's tau protein in the brain of
the transgenic animals. These animals exhibit NF pathological

activity in brains. The invention further relates to a transgen-
ic animal, which can be used as a screening assay system in the
process of identifying substances, which improve learning and
memory, inhibit neurodegeneration and decrease the rate of neu-
rofibrillary tangles (NFT) formation and their elimination from
the brain. The animals can be used for identifying substances
for prevention, treatment and diagnosis of neurodegenerative
diseases, preferably tauopathies and AD. The transgenic animals
can also be used for investigating the influence of AD associ-
ated risk factors, on the development of AD. Another use of an-
imals lies in the process of drug target identification.

The present invention describes the engineering of transgenic
animals producing AD changes induced by specific transgene, re-
sembling Alzheimer's disease in these animals. This provides an
investigative tool to the discovery and development of drugs and
diagnosis and to further study the role of normal and diseased
tau proteins in vivo.

The present invention relies on an induction of NF pathological
activity in brain of animals solely by expressing of Alzheimer's
tau protein in the brains of transgenic animals. Hallmarks of AD
are induced in animals, which are prone to induction of risk
factors associated with etiology of AD, which is another advant-
age of the present invention.

All technical and scientific terms used in the description of
preferred embodiments have - unless specified otherwise - the
same meaning as commonly understood in the field of art to which
this invention belongs. The preferred methods, the laboratory
procedures in cell culture, molecular genetics, biochemistry,
and nucleic acid chemistry and materials are described in the
invention, but any methods and materials similar or equivalent
can be used in testing of the present invention. For procedures
like recombinant nucleic acid preparation, biochemical analysis,
cell culture, and transgene incorporation like electroporation,
lipofection and microinjection standard techniques are used.

The term "Alzheimer's tau" refers to the group of specifically
truncated isoforms of tau protein engineered genetically that

corresponds to those present exclusively in AD diseased brain. Alzheimer's tau is capable of inducing of NF activity on its own or in combination of other molecules in the brain. As used herein, the term Alzheimer's tau protein refers to the group of truncated tau forms present in AD-diseased human brain tissue.

The term "transgene" is used herein to describe genetic material, which has been artificially inserted into the genome of a cell, particularly a mammalian cell for implantation into a living animal. For specific countries, it may be necessary to specifically exclude certain subject matter from this aspect, such as human totipotent stem cells as such or processes which lead to such cells, falling under Art.6 (1) and (2) of the EC Directive 98/44/EC.

By "Alzheimer's disease" (abbreviated herein as "AD") is meant a condition associated with formation of neurofibrillary tangles and beta amyloid plaques, as well as impairment in both learning and memory. "AD" as used herein is meant to encompass both AD as well as AD-type pathologies, i.e., diseases of the central nervous system with symptoms similar to AD.

By "Neurofibrillary activity" (abbreviated herein as "NF" activity) is meant a condition associated with formation of neurofibrillary tangles in central nervous system with symptoms similar to AD.

By "symptoms similar to AD" and "phenomenon associated with AD" is meant a structural, molecular, or functional event associated with AD, particularly such an event that is readily assessable in an animal model. Such events include, but are not limited to, neurofibrillary pathology, learning and memory deficits, and other AD-associated characteristics.

By "transgenic animal" is meant a non-human animal, usually a mammal (e.g., a mouse, rat, rabbit, hamster, etc.), having a non-endogenous (i.e., heterologous) nucleic acid sequence present as an extrachromosomal element in a portion of its cell or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic

acid is introduced into the germ line of such transgenic animals
by genetic manipulation of, for example, embryos of the host an-
imal.

By "construct" is meant a recombinant nucleic acid sequence,
generally recombinant DNA sequences, operable linked to tissue
specific or general promoter, which is generated for the purpose
of the expression of a specific nucleotide sequence(s), in mam-
malian cells, or is to be used in the construction of other re-
combinant nucleotide sequences.

The present invention provides the structure, sequence and meth-
od for generating of DNA constructs used for transgenic animal
preparation, which are characterised by the following features:

A DNA construct comprises a cDNA molecule coding for N- and C-
terminally truncated tau molecules, wherein
- the molecules have truncated at least 30 nucleotides down-
  stream of the start codon and truncated at least the 30 nucle-
  otides upstream of the stop codon of the full length tau cDNA
  sequence coding for 4-repeat and 3-repeat tau protein, re-
  spectively, as given in Seq.accession number NM_173727 in
  GeneBank
- the minimally truncated tau core encompasses a protein frag-
  ment which is encoded by nucleotides nos 744 - 930 (seq ID No.
  9)(numbered according to tau protein isoform 43)
- said DNA constructs are coding for proteins, which have neur-
  ofibrillary (NF) pathology producing activity when expressed
  in brain cells of animals.

  This NF pathology producing activity can be quantified by
  counts of neurofibrillary tangles per selected brain region,
  e.g. using stereology approach. Alternatively, neurobehaviour-
  al tests can be used for reliable evaluation of cognitive
  scores in learning memory-addressing tasks.

Specifically preferred tau cDNA molecules according to the
present invention comprise a nucleotide sequence selected from
the group of SEQ ID NOs 1 to 14. The sequences are depicted in
Fig. 1.

In a preferred embodiment of the invention, said cDNA molecules represent a group of N- and C-terminally double truncated cDNAs, which comprises the following nucleotide sequences: (Derivatives from 4-repeat tau (tau 43) are labeled R4 and derivatives from 3-repeat tau (tau 43) are labeled R3). Numbering used here is derived from full length tau cDNA described in Goedert et al.(24):

SEQ ID NO: 1 (91-1059, R4)
ATGCACCAAGACCAAGAGGGTGACACGGACGCTGGCCTGAAAGCTGAAGAAGCAGGCATTG-
GAGACACCCCCAGCCTGGAAGACGAAGCTGCTGGTCACGTGACCCAAGCTCGCATGGT-
CAGTAAAAGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATG-
GTAAAACGAAGATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGC-
CAACGCCACCAGGATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGT-
GAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCG-
GCAGCCGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTG-
GCAGTGGTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGC-
CCCCGTGCCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCT-
GAAGCACCAGCCGGGAGGCGGGAAGGTGCAGATAATTAATAAGAAGCTGGATCT-
TAGCAACGTCCAGTCCAAGTGTGGCTCAAAGGATAATATCAAACACGTCCCGGGAGGCG-
GCAGTGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCT-
CATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCT-
TGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTG-
GCGGAGGAAATAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGCCAAAGC-
CAAGACAGACCACGGGGCGGAGATCGTGTACAAGTCGCCAGTGGTGTCTGGG-
GACACGTCTCCACGGCATCTCAGCAATGTC

SEQ ID NO: 2 (205-999, R4)
ATGGTCAGTAAAAGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATG-
GTAAAACGAAGATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGC-
CAACGCCACCAGGATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGT-
GAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCG-
GCAGCCGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTG-
GCAGTGGTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGC-
CCCCGTGCCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCT-
GAAGCACCAGCCGGGAGGCGGGAAGGTGCAGATAATTAATAAGAAGCTGGATCT-
TAGCAACGTCCAGTCCAAGTGTGGCTCAAAGGATAATATCAAACACGTCCCGGGAGGCG-
GCAGTGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCT-
CATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCT-

TGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTG-
GCGGAGGAAATAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGCCAAAGC-
CAAGACAGACCACGGGGCGGAG

SEQ ID NO: 3 (277-999,R4)
ATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGCCAACGCCACCAG-
GATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGTGAACCTCCAAAAT-
CAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCGGCAGC-
CGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTGGCAGTG-
GTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGCCCCCGTGC-
CCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCTGAAGCACCAGC-
CGGGAGGCGGGAAGGTGCAGATAATTAATAAGAAGCTGGATCTTAGCAACGTCCAGTCCAAGT-
GTGGCTCAAAGGATAATATCAAACACGTCCCGGGAGGCGGCAGTGTGCAAATAGTCTACAAAC-
CAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAG-
GAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCTTGACTTCAAGGACA-
GAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTGGCGGAG-
GAAATAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGCCAAAGCCAAGACAGAC-
CACGGGGCGGAG

SEQ ID NO: 4 (205-1089,R4)
ATGGTCAGTAAAAGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATG-
GTAAAACGAAGATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGC-
CAACGCCACCAGGATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGT-
GAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCG-
GCAGCCGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTG-
GCAGTGGTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGC-
CCCCGTGCCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCT-
GAAGCACCAGCCGGGAGGCGGGAAGGTGCAGATAATTAATAAGAAGCTGGATCT-
TAGCAACGTCCAGTCCAAGTGTGGCTCAAAGGATAATATCAAACACGTCCCGGGAGGCG-
GCAGTGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCT-
CATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCT-
TGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTG-
GCGGAGGAAATAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGCCAAAGC-
CAAGACAGACCACGGGGCGGAGATCGTGTACAAGTCGCCAGTGGTGTCTGGG-
GACACGTCTCCACGGCATCTCAGCAATGTCTCCTCCACCGGCAGCATCGACATGGTAGAC

SEQ ID NO: 5 (277-1089,R4)
ATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGCCAACGCCACCAG-
GATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGTGAACCTCCAAAAT-
CAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCGGCAGC-

CGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTGGCAGTG-
GTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGCCCCCGTGC-
CCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCTGAAGCACCAGC-
CGGGAGGCGGGAAGGTGCAGATAATTAATAAGAAGCTGGATCTTAGCAACGTCCAGTCCAAGT-
GTGGCTCAAAGGATAATATCAAACACGTCCCGGGAGGCGGCAGTGTGCAAATAGTCTACAAAC-
CAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAG-
GAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCTTGACTTCAAGGACA-
GAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTGGCGGAG-
GAAATAAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGCCAAAGCCAAGACAGAC-
CACGGGGCGGAGATCGTGTACAAGTCGCCAGTGGTGTCTGGGGACACGTCTCCACGGCATCT-
CAGCAATGTCTCCTCCACCGGCAGCATCGACATGGTAGAC

SEQ ID NO: 6 (715-999,R4)
ATCAAACACGTCCCGGGAGGCGGCAGTGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAG-
GTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTG-
GAAGTAAAATCTGAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTG-
GACAATATCACCCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCT-
GACCTTCCGCGAGAACGCCAAAGCCAAGACAGACCACGGGGCGGAG

SEQ ID NO: 7 (709-999,R4)
GATAATATCAAACACGTCCCGGGAGGCGGCAGTGTGCAAATAGTCTACAAACCAGTTGACCT-
GAGCAAGGTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGC-
CAGGTGGAAGTAAAATCTGAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGG-
GTCCCTGGACAATATCACCCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCT-
GACCTTCCGCGAGAACGCCAAAGCCAAGACAGACCACGGGGCGGAG

SEQ ID NO: 8 (715-954,R4)
ATCAAACACGTCCCGGGAGGCGGCAGTGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAG-
GTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTG-
GAAGTAAAATCTGAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTG-
GACAATATCACCCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCTG

SEQ ID NO: 9 (741-930,R4)
GTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCTCATTAG-
GCAACATCCATCATAAACCAGGAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCT-
TGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCACCCACGTCCCTG-
GCGGAGGAAAT

SEQ ID NO: 10 (91-966,R3)

ATGCACCAAGACCAAGAGGGTGACACGGACGCTGGCCTGAAAGCTGAAGAAGCAGGCATTG-
GAGACACCCCCAGCCTGGAAGACGAAGCTGCTGGTCACGTGACCCAAGCTCGCATGGT-
CAGTAAAAGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATG-
GTAAAACGAAGATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGC-
CAACGCCACCAGGATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGT-
GAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCG-
GCAGCCGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTG-
GCAGTGGTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGC-
CCCCGTGCCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCT-
GAAGCACCAGCCGGGAGGCGGGAAGGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGT-
GACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTG-
GAAGTAAAATCTGAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTG-
GACAATATCACCCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCT-
GACCTTCCGCGAGAACGCCAAAGCCAAGACAGACCACGGGGCGGAGATCGTGTACAAGTCGC-
CAGTGGTGTCTGGGGACACGTCTCCACGGCATCTCAGCAATGTC

SEQ ID NO: 11 (205-906,R3)
ATGGTCAGTAAAAGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATG-
GTAAAACGAAGATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGC-
CAACGCCACCAGGATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGT-
GAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCG-
GCAGCCGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTG-
GCAGTGGTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGC-
CCCCGTGCCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCT-
GAAGCACCAGCCGGGAGGCGGGAAGGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGT-
GACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTG-
GAAGTAAAATCTGAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTG-
GACAATATCACCCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCT-
GACCTTCCGCGAGAACGCCAAAGCCAAGACAGACCACGGGGCGGAG

SEQ ID NO: 12 (277-906,R3)
ATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGCCAACGCCACCAG-
GATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGTGAACCTCCAAAAT-
CAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCGGCAGC-
CGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTGGCAGTG-
GTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGCCCCCGTGC-
CCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCTGAAGCACCAGC-
CGGGAGGCGGGAAGGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGTGACCTCCAAGT-
GTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTGGAAGTAAAATCT-
GAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCAC-

CCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGC-
CAAAGCCAAGACAGACCACGGGGCGGAG

SEQ ID NO: 13 (205-996,R3)
ATGGTCAGTAAAAGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATG-
GTAAAACGAAGATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGC-
CAACGCCACCAGGATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGT-
GAACCTCCAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCG-
GCAGCCGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTG-
GCAGTGGTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGC-
CCCCGTGCCCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCT-
GAAGCACCAGCCGGGAGGCGGGAAGGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGT-
GACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTG-
GAAGTAAAATCTGAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTG-
GACAATATCACCCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCT-
GACCTTCCGCGAGAACGCCAAAGCCAAGACAGACCACGGGGCGGAGATCGTGTACAAGTCGC-
CAGTGGTGTCTGGGGACACGTCTCCACGGCATCTCAGCAATGTCTCCTCCACCG-
GCAGCATCGACATGGTAGAC

SEQ ID NO: 14 (277-996,R3)
ATCGCCACACCGCGGGGAGCAGCCCCTCCAGGCCAGAAGGGCCAGGCCAACGCCACCAG-
GATTCCAGCAAAAACCCCGCCCGCTCCAAAGACACCACCCAGCTCTGGTGAACCTCCAAAAT-
CAGGGGATCGCAGCGGCTACAGCAGCCCCGGCTCCCCAGGCACTCCCGGCAGC-
CGCTCCCGCACCCCGTCCCTTCCAACCCCACCCACCCGGGAGCCCAAGAAGGTGGCAGTG-
GTCCGTACTCCACCCAAGTCGCCGTCTTCCGCCAAGAGCCGCCTGCAGACAGCCCCCGTGC-
CCATGCCAGACCTGAAGAATGTCAAGTCCAAGATCGGCTCCACTGAGAACCTGAAGCACCAGC-
CGGGAGGCGGGAAGGTGCAAATAGTCTACAAACCAGTTGACCTGAGCAAGGTGACCTCCAAGT-
GTGGCTCATTAGGCAACATCCATCATAAACCAGGAGGTGGCCAGGTGGAAGTAAAATCT-
GAGAAGCTTGACTTCAAGGACAGAGTCCAGTCGAAGATTGGGTCCCTGGACAATATCAC-
CCACGTCCCTGGCGGAGGAAATAAAAAGATTGAAACCCACAAGCTGACCTTCCGCGAGAACGC-
CAAAGCCAAGACAGACCACGGGGCGGAGATCGTGTACAAGTCGCCAGTGGTGTCTGGG-
GACACGTCTCCACGGCATCTCAGCAATGTCTCCTCCACCGGCAGCATCGACATGGTAGAC

The truncated tau sequences may be adapted due to the specific
host cell whereto the construct is transferred (codon usage,
codon preferences, homolog adaptations (exchanging one or more
of the human codons with the host cell codon, if differences are
present).

Preferred features of the constructs:

The above cDNA sequences are linked to regulatory sequences in order to drive an expression of truncated tau protein in the brain. Modification of Thy-1 gene (25) was performed in such a way that regulatory sequence, which directs an expression into T-cells was deleted and truncated cDNA sequence was introduced in between Spe-I and Xho-restriction sites. The modified construct was stripped of prokaryotic sequences and introduced into male pronucleus via microinjection. Any functional promoters or promoter-enhancer complexes may be used for expression of above described truncated tau cDNAs.

According to another aspect, the present invention provides a method for the preparation and testing of molecules according to the present invention, characterized by the following steps:

(a) construction of a recombinant prokaryotic cloning plasmids carrying coding sequences for a double truncated tau molecule with deletions covering at least the first first 30 and the last 30 nucleotides of three or four repeat tau cDNA molecules,

(b) construction of an eukaryotic expression plasmid carrying appropriate promoters for brain expression or ubiquitous expression and respective cDNA sequences of truncated tau molecules and the combination thereof,

(c) growing bacteria containing said plasmids and amplification, of the plasmids and their extraction in high quantity,

(d) transfection of said gene constructs into COS-7 cells and their analysis using western blot technique,

(e) isolation and purification of the gene construct such that all prokaryotic sequences are excluded, and dilution of transgenes in a buffer in concentration suitable for microinjection (see Example 1),

(f) verification of gene constructs intended for microinjection.

This is performed by restriction analysis, gene sequencing and protein analysis after transient transfections into mammalian cells after which the correct size of proteins and their reactivity with specific monoclonal antibodies were evaluated (see Example 1, Fig. 2.b).

The present invention therefore provides a transgenic non-human animal of whose germ and/or somatic cells comprise the DNA construct encoding for Alzheimer's tau protein, which can be used as a suitable animal model of Alzheimer's disease.

It is therefore an object of the present invention to provide a transgenic non-human animal all of whose germ and/or somatic cells comprises the human truncated tau cDNA molecules. Cloned human Alzheimer's tau cDNA sequence is linked to a promoter sequence, which directs the expression of Alzheimer's tau in tissue specific manner or ubiquitously.

Transgenic animals of the invention are engineered using well known regular procedures of microinjection, into the male pronucleus of one day old fertile embryos of rats. The transgenes can be introduced into embryonic cells also by other methods known in the art, including transfection, lipofection, electroporation and with the help of retroviruses or by other means. Embryos carrying the transgene can then be implanted into pseudopregnant animals.

It is therefore an object of invention to provide a method for genotyping of newborn animals. PCR analysis using specific oligonucleotides that allow an amplification of targent sequences of the transgene is therefore employed for this purpose. Oligonucleotides used for genotyping were designed in a way that the transcription initiation site of the transgene including the ATG codon and Kozak sequence could be verified eventually by DNA sequencing of the PCR product (primer pair A). Moreover one primer was anneals to the heterogeneous promoter and another primer anneals to nucleotide sequence coding for Alzheimer's tau protein. Similar designing criteria were applied for the stop codon (primer pair B). Integrity of the transgene therefore can be monitored this way. Sequences of the diagnostic oligonucleotides are therefore as follows:

Primer pair A:
Sense: 5'-GTGGATCTCAAGCCCTCAAG-3'
Antisense: 5'-GATCCCCTGATTTTGGAGGT-3'
Expected size of PCR product is 235 nucleotides.

Primer pair B
Sense: 5'- AAGGTGACCTCCAAGTGTGG- 3'
Antisense: 5'- GGTATGCATGGAGGGAGAAG- 3'
Expected size of PCR product is 438 nucleotides.

Presence of the PCR fragment is therefore specific to animals
with transgene integrated in the genome (see Example 2)

The founder animals can be used for breeding with wild-type an-
imals to produce F1 generation of animals, which are heterozyg-
ous for the transgene. In further embodiments, these heterozyg-
ous animals can be interbred to obtain the viable transgenic em-
bryos whose somatic and germ cells are homozygous for the genes
coding for Alzheimer's tau protein.

Another object of the present invention is to provide non-human
animals of which germ and somatic cells transiently or stably
express said DNA construct coding for Alzheimer's tau protein
and exhibiting NF pathology in the brain. The preferred animal
of this invention is a rat, wherein the protein encoded by said
DNA molecules is expressed in the brain.

The most preferred embodiment of the present invention is a
transgenic non-human animal wherein the Alzheimer's tau protein
encoded by said DNA molecules is expressed in the brain of the
animal. The expression of Alzheimer's tau was detected by sever-
al monoclonal antibodies as described in Example 3 and docu-
mented on Fig. 3. Expression of the protein in different brain
regions of transgenic lineage is documented on Fig. 4.

The present invention therefore provides a transgenic non-human
animal wherein DNA molecules coding for Alzheimer's tau protein
are stably integrated in the genome of said animals or otherwise
present in the cell nucleus in order to be transcribed. Said an-
imals are characterized by the following features:
- transgenes are transmitted to subsequent generations of anim-
  als according to Mendelian laws forming a transgenic lineage
  (see Example 4)
- the inherited transgene is expressing truncated proteins in

brains of the founder animals and the progeny as shown in Ex-
ample 3 Fig. 4.

The present invention therefore provides the transgenic non-hu-
man animals, which exhibit neurofibrillary pathology in the
central nervous system (CNS). Said animals are characterized by
the following features:
- Expression of truncated tau proteins promotes the formation of
  neurofibrillary pathology (Example 5)
- PHF 1 immunoreactive NFTs showed that endogenous normal rat
  tau participate on the formation of NFTs (Example 5)
- Neurofibrillary tangles are present as intracellular inclu-
  sions and filaments in the neurons of CNS and are homologous
  to NFT in human AD diseased brains.
- Immunofluorescence revealed the presence of small – rod like
  structures similar to those observed in pre- clinical
  Alzheimer´s disease – observed in human brain tissue (see Ex-
  ample 5).

In the preferred embodiment of the invention, transgenic animals
display such neuropathology as neurofibrillary tangles, ghost
tangles and neuropil threads that are formic acid sensitive.
Neurofibrillary tangles are composed of an abnormal accumulation
of intraneuronal filaments. The tangle components are comprised
from Alzheimer´s tau as well as normal tau molecules. Such
transgenic animals serve as suitable model system for study of
Alzheimer´s disease and development of therapeutic, preventive
and diagnostic substances.

The invention provides a transgenic animal developing NF patho-
logy, and having a genetic background allowing induction of risk
factors associated with AD, thereby representing a disease model
for humans.

The present invention therefore provides a transgenic non-human
animal, wherein the genetic background, in which DNA molecules
are stably integrated in the genome of spontaneously hypertens-
ive animal or otherwise present in the cell nucleus in order to
be transcribed within the specific genetic background.

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Another aspect of the invention is to provide an animal model
comprising the combination of genetic factors and other risk
factors associated with AD as follows:
- hypertension which is the most common risk factors of AD,
- diabetes which can be induced by diet (diabetes is important
  risk factor of AD),
- hypercholestrolemia which can be induced by diet (another im-
  portant risk factor of AD).

Said pathological situations can easily be induced in the
provided animal model. Suitable examples were previously repor-
ted in literature (e.g. in 26, 27, 28).

Moreover the provided animal model is the first animal model,
which expresses neurofibrillary pathology on the genetic back-
ground of SHR.

According to another aspect the present invention provides an
animal, which represents an experimental model of sporadic
Alzheimer's disease, said model permits an assessment of the
role of risk factors associated with AD development. Risk
factors play a critical role in the vast majority of AD cases,
the model may therefore be considered as a model of sporadic AD
which represents 90% of all cases of AD neurodegenerations.

In another preferred embodiment, the present invention provides
a screening assay system for substances for the treatment pre-
vention and diagnosis of Alzheimer's disease, which comprises:
- evaluation of substances by:
    • detecting changes of neurofibrillary pathology in said an-
      imals
    • measuring of neurobehavioural changes in transgenic anim-
      als
    • measuring of the cognitive score in transgenic animals
    • biochemical measuring of AD specific markers in animal
      tissues and body fluids
- a validation system for substances for the treatment and pre-
  vention of tauopathies preferably AD
- a validation system for the development of diagnostic markers
  and probes for the detection tauopathies, preferably AD

- a validation system for substances for treatment of hyperten-
  sion, diabetes, dislipidaemia and hypercholesterolemia in com-
  bination with tauopathies preferably AD

The present invention therefore provides an in-vivo assay to as-
sess the efficacy of substances, or therapies in particular NFT
reducing or preventing therapies. The animals can be used for
screening of substances or therapies for neurodegenerative dis-
eases, in particular tauopathies, preferably AD and other neuro-
degenerative diseases accompanied by neurofibrillary tangles
formation.

Therefore the present invention provides a model system consist-
ing of transgenic animals, cells and assays that are useful in
the study of the etiology and treatment of Alzheimer's disease.
The assays are also useful for screening for substances that in-
hibit the formation of neurofibrillary pathology and other
therapeutic effects of the substances.

The transgenic animals of the present invention and animal cells
derived thereof are used for screening compounds for a potential
effect in the treatment of Alzheimer's disease using standard
methodologies. The compound is administered to the animals or
introduced into the culture media over a period of time and in
various dosages, then the animals or animal cells are examined
for alterations in histopathology and Alzheimer's tau expression
respectively. Furthermore, the ability to perform neurobehavi-
oural testing to the transgenic animals enables cognitive func-
tion to be monitored following treatment with potential thera-
peutic agents. Screening of compounds leads to the selection of
specific agents for the prevention or treatment or diagnosis of
Alzheimer's disease. Many variations of screening methods are
known to those skilled in the art and may be applied within the
scope of the present invention.

The transgenic animal model of the present invention may also be
used to unravel the molecular cascade of events leading to
neurodegeneration and Alzheimer's disease such that therapeutic
targets may be identified.

Still another aspect of the invention is to provide an in vitro
assay based on cell lines derived from a transgenic animal or
cell line derived from a transgenic rat embryo of the present
invention, where the said assay is employed as a screening and
validation tool for the discovery and development of Alzheimer's
diagnosis and markers. Said in vitro assay is based on the cell
lines and may also be employed as a screening and validation
tool for the discovery of anti Alzheimer's therapeutic and pre-
ventive drugs.

The following examples are presented by the way of illustration
and, because various other embodiments will be apparent to those
in the art, the following is not to be construed as a limitation
on the scope of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1
Scheme of Alzheimer's tau protein cDNAs employed for engineering
of gene expression constructs, which were used in production of
transgenic animals of the present invention.

Fig. 2 Preparation and verification of gene constructs used for
the production of transgenic animals. Construction of transgenic
animals was performed via pronuclear microinjection of cloned
gene constructs containing coding sequence for Alzheimer's tau.
Gene constructs were verified by restriction analysis and PCR
using transgene specific primers (panel A, agarose gel of PCR
amplified and DNA fragment) as well as by DNA sequencing in or-
der to control for potential mutations. Verification was fol-
lowed by purification of gene constructs (panel B, agarose gel
of pure transgene fragment) and transient transfections into
mammalian cells (panel C, Western blot verification of protein
expression). After the assays were completed the constructs were
stripped of prokaryotic sequences and injected into one -day old
rat embryos. The bottom flow chart shows a simplified procedure
of transgenic animals production.

Fig. 3 Genotyping of transgenic founder animals and F1 genera-
tion of transgenic animals. Panel "A" shows controls for the

genotyping experiment (+C, positive control; NAC, no amplifica-
tion control; NTC, no template control). Panel "B" shows results
of PCR genotyping. Sample number 12 represents a transgene pos-
itive animal Panel "C" outlines the breeding experiments per-
formed to confirm founder animals. Furthermore the panel shows
transgenic line of the present invention that has transmitted
the transgene to the subsequent generations according to
Mendelian lows.

Fig. 4
Expression of Alzheimer's tau protein in brain of transgenic
rat. The left panel represents a Western blot analysis and right
panel is presenting total amount of proteins loaded per lane.
Alzheimer's tau protein migrates at the size of 35kD (lane 1,
arrow), detected by pan-tau monoclonal antibody DC25. For West-
ern blot an ECL technique was employed, and specific signal was
observed after 1min exposure. The 7.5 ug of total protein ex-
tract from brain lysates of transgenic animal was loaded (lane
1) whereas 25 ug of proteins was loaded from
wild type control animals. M, molecular size marker.

Fig. 5
Expression of Alzheimer's tau protein in different brain regions
of transgenic rat. Western blot analysis of total protein ly-
sates from transgenic rat brain regions. Lane 1, complete brain
lysate of founder animal (generation F0) - 10ug proteins; lane
2, total brain lysate of transgenic animal of F2 generation;
lanes 3-6, protein lysates from transgenic animal of F2 genera-
tion: 3 - hippocampus, 4 - subcortical area, 5 - cerebral cor-
tex, 6 - spinal cord and medulla; lane 7- 8 represent brain ly-
sates from control non-transgenic littermates of F2 generation:
7 - cortex, 8 - cerebellum (Total protein load per lanes 2-8 was
20ug).

Fig. 6
Silver staining of brain sections from transgenic animal and non
- transgenic control wild type animal. Intracellular inclusions
and neurofibrillary filaments were detected in the neurons of
the CNS of transgenic rat, (panel B and C, transgenic animal).
Wild type rats do not show these structures in the homologous

brain area (A, wild type control). Magnification: 200x (A and B), 400 x (C)

Fig. 7

Detection of neurofibrillary tangles using the pan-tau mono-
clonal antibody DC 25 in central nervous system of transgenic
animal. mAb DC25 recognizes NFTs in the neurons of AD patients.
The equivalent structures are recognized in the brain of trans-
genic rat (panel B and D). Wild type rats did not show any sim-
ilar structures in the homologous brain area, (A, C). Magnifica-
tion: 100x (A,B), 200x (C, D)

Fig. 8

Detection of neurofibrillary tangles by monoclonal antibody PHF-
1 in central nervous system of the transgenic animal. PHF-1 is
specific marker for tau phosphorylation. Immunoreactivity with
this antibody shows that expression of Alzheimer's tau involves
endogenous tau in the process of formation of NFTs. Furthermore,
immunofluorescent staining shows small rod like structures sim-
ilar to those observed in pre-clinical Alzheimer's disease brain
tissues. Magnification: 100x (A), 200x (B), 1000x (C, D)

Fig. 9

Neuronal cell culture as a screening assay system for character-
ization of therapeutic leads and drug candidates. Primary cul-
tures of transgenic and nontransgenic rat neurons can be used
for this purpose after in vitro cultivation and effect of the
drug candidates on the mitochondrial transport can be evaluated
using videomicroscopy. Cortical (Cx) and and hippocampal (Hipp)
neurons loaded with vital dye (e.g. Mito-trek) are shown after 3
days in vitro cultivation (3DIV). Mitochondria (MT) are visual-
ized.

Fig. 10

Histophathological features of Alzheimer's disease, recapitu-
lated in transgenic animal of present invention. Presented neu-
rofibrillary pathology is one of the major hallmark of
Alzheimer's disease. Fig. 10 shows a comparison of neurofibril-
lary tangles detected by Gallyas silver technique (A, C) and
also by immunohistochemistry (E) in AD diseased human brain, to

the equivalent pathological structures observed in transgenic
animal of present invention. Immunochemical staining was per-
formed with monoclonal antibody 7.51 which recognizes generic
tau epitope in the tandem repeat region (29).

E X A M P L E S

**EXAMPLE 1: Design of gene constructs containing DNA sequence
coding for Alzheimer's tau protein - human truncated tau gene;
engineering and verification of gene constructs for ubiquitous
and tissue specific expression in transgenic animals.**

Construction of Alzheimer's tau expression vectors consist of
procedures that include:
- design of coding sequence especially deletions and truncations
  are shown in 1.
- these portions of tau gene were PCR amplified with the help of
  proofreading DNA polymerase and oligonucleotides equipped with
  appropriate restriction sequences, so that they can be cloned
  under general or tissue specific promoters in an eukaryotic
  expression vector, cloning was performed using standard pro-
  cedures in a common bacterial strains
- verification of cloned genes was performed in order to check
  directionality of the inserted gene which was confirmed by re-
  striction digestions and or PCR analysis (Fig. 2A). In addi-
  tion, the constructs were partially sequenced to exclude the
  possible mutated forms
- purification of fragment intended for microinjection (Fig. 2B)
- verification of protein expression using transfections of
  transgene into mammalian cells, mainly COS-7 and C6 rat glioma
  cells, and analysis of expression of proteins in a eukaryotic
  cells by western blot method (Fig. 2C).

All the methods are described in a manual: Sambrook et al., Mo-
lecular Cloning, A Laboratory Manual, Cold Spring Harbor Labor-
atory Press, 1989 (14).

EXAMPLE 2: Production of transgenic rat

Cloned gene construct containing Alzheimer's tau coding sequence

was injected into male pronucleus of one day old embryos. Fer-
tilized eggs were extracted from SHR donor females in separate
experiments using routine techniques (15). After microinjecting
of DNA, the zygotes were in vitro cultured in CO_2 incubator for
about 12 hours and implanted into oviductus of pseudopregnant
foster mothers. Founders were identified by PCR of transgene
specific target sequence from genomic DNA. To determine the tau
genotypes, genomic DNA was purified from about 0.5 cm of tail
taken from each rat. PCR analysis, was used to identify off-
spring which contained the transgene. Oligonucleotides for dia-
gnostic PCR screening were designed in such a way that integrity
of the transgene could be easily determined. For determination
of number of transgene copies integrated in rat genome a real
time PCR analysis was used.

For example Tg line #318 contained 4 copies of the transgene,
which was determined by real time PCR using Sybre Green method
and BioRad PCR machine.

**EXAMPLE 3: Genotyping of animals born after embryo implantation
into foster mothers, identification of transgenic animals and
assessment of transmission of the transgene to subsequent gener-
ations**

DNA extracted from tail tips: Genomic DNA was extracted by
DNeasy tissue kit, Qiagen.
Genotyping: Specific amplification of transgenes encoding double
truncated tau forms was performed on genomic DNA derived from
the parental generation of transgenic animals and is shown in
Figure 3A and 3B. Further analysis of genomic DNA of the F1 gen-
eration revealed that transgenes are heritable since they were
also identified in the offspring of parental generation. Trans-
genes encoding Alzheimer's tau are therefore fixed in chromo-
somal DNA of the animals. Genotyping was performed with two pos-
itive (plasmid and TG positive genomic DNA) and two negative
controls (no amplification control heterologous genomic DNA; no
template control for control of chemicals) as it is shown in
Fig. 3A.

Analysis of transgene expression: Expression of mRNA derived

from the transgenes were assessed by RT-PCR analysis, applying
generally known methods including RT-PCR and agarose gel elec-
trophoresis. RNA was extracted from flash frozen tissue of
transgenic animals and subjected to reverse transcription fol-
lowed by specific amplification of the cDNA.

Rat breeding and generation of heterozygous and homozygous rats:
Transgene positive rats were bred to wild-type SHR.Germline
transmission was achieved in several lines. In this example Tg
line #318 is shown. Male and female transgenic rat, each of
which contained four copies of the truncated human tau protein
gene were mated with each other and wild type SHR rat to expand
transgenic colony. Mendelian splitting ratios of transgene in
the offspring were observed (Fig. 3C).

The transgenic animals of the present invention can be used to
cross breed with transgenic animals overexpressing the human APP
containing familial Alzheimer's disease (FAD) mutation or PS1
(13). The resulting rat will express both APP and PS1 with or
without FAD mutation on hypertensive genetic background. The ef-
fect of PS1 and APP on NFT formation can be studied both in vivo
and in vitro. Specifically, the onset and severity of AD-related
pathologies can be examined by means of immounhistochemistry.

**EXAMPLE 4: Expression of Alzheimer's tau protein in the brain of
transgenic rats**

Protein expression was performed using western blot analysis
employing tau protein specific monoclonal antibodies. We have
analysed brain tissues of rats that were geneticaly positive for
transgene and were able to transmit the transgene to subsequent
generations. Experiments were controled by simultaneous analysis
of brain extracts from transgene negative age matched rats and
transgene negative littermates.

Tissue sample preparation: approximately 2mg of freshly isolated
brain tissue was mechanically homogenized in 30µl modified Hunt's
lysis buffer (20mM Tris, pH 8.0, 100mM NaCl, 1mM EDTA, 0.5% Ige-
pal CA630, 0.5% Triton X-100), which contained protease inhibit-

or mix Complete-EDTA free (Roche). 170µl of lysis buffer were ad-
ded, homogenate was triturated with syringe and needle (22G) and
frozen in liquid nitrogen. After slow thawing on ice, lysate was
sonicated and centrifuged 15 minutes, 10000g at +4°C. Protein
content in samples was determined by Bradford assay using Beck-
man spectrophotometer.

PAGE and Western blot. Proteins were separated on 12% denaturing
polyacrylamid gel with 0.1% (w/V) SDS. Electrophoresis was per-
formed in Tris-glycin buffer (25mM Tris, 192mM glycin, 0.1%
SDS). Protein separation was followed by semi-dry blotting.
Tris-glycin-isopropanol buffer system (25mM Tris, 192mM glycin,
20 % Isopropanol) was used. Tau proteins were stained with anti-
tau monoclonal antibodies DC25 which recognize tau proteins
equally as a mAb 7.51 (29) and also with mAb Tau-1 (21). The
positive signal was determined using anti-mouse HRP antibody and
ECL method. Efficiency of blotting procedure was controled by
Ponceau staining. Protein analysis has revealed the specific ex-
pression of Alzheimer's tau protein in total protein extract of
brain of transgenic rat (Fig. 4) and also an expression of
Alzheimer's tau protein in different brain regions of transgenic
rat (Fig. 5).

**EXAMPLE 5: Immunohistochemical analysis of brain of transgenic
rat.**

In order to perform immunohistochemical analysis of transgenic
rat, several tau protein and Alzheimer specific monoclonal anti-
bodies were used. Short description of monoclonal antibodies em-
ployed in these studies include PHF 1, which recognizes phos-
phorylated epitope Ser 396 - Ser 404; mAb DC 25 recognizes phos-
phate independent epitope Lys347 - Ile 353; mAb AT 8 recognizes
phosphorylated epitope Ser 198 - Ser 202; mAb Tau 1 recognizes
non- phosphorylated epitope, mAb Tau 5 recognizes phosphate
independent epitope. (15, 16, 17, 18, 19, 20, 21, 22)

Light microscopy and immunofluorescence:
Tissues were prepared and sectioned as follows. Transgenic and
control animals were anaesthetized with ether and immediately
perfused intracardially with buffered 4 % paraformaldehyde,

brains were removed, cryoprotected with 20 % sucrose, quickly
frozen and sectioned by cryostat at 50 μm thickness. Some of the
sections were shortly pretreated with 70 % formic acid prior
staining procedure. Rat brain tissue sections were incubated
with 1 % H_2O_2 in PBS and non- specific binding sites were
blocked with incubation in blocking medium (PBS, 0, 1 % Triton,
5 % normal horse serum). The immunostaining was performed using
antibodies listed above, then incubated with biotinylated horse
anti- mouse antibody, followed by avidin- biotin complex and
visualized with VIP and DAB solution. Tissue sections were moun-
ted on gelatin- coated slides, dried, treated with graded alco-
hols, xylene and coverslipped with Entellan.

Silver staining:
Gallyas silver staining method that has been described previ-
ously (23) was adapted for free floating 50 μm sections, with
gold toning.

Immunofluorescent staining:
For indirect immunofluorescent labeling, sections were pre-
treated with 1 % $NaHBO_4$ for 30 min. to reduce brain tissue auto-
fluorescence. Then stained with primary antibodies at 4 °C
overnight, incubated with a horse anti mouse biotinylated anti-
body, followed by streptavidin- Alexa 488 conjugate.

Comparison of NF pathology as seen in the brains of patients
suffering from AD with those observed in the brain of transgenic
animal of present invention is represented in Fig. 10.

EXAMPLE 6: A screening assay systems and for drug leads and drug
candidates for the treatment and prevention of Alzheimer's dis-
ease

The transgenic animals of the present invention can be used as a
source of cells for cell culture. Both animals and cells can be
used in assays of this invention. For example, cells of brain
tissues expressing truncated tau gene can be cultured using
standard culture techniques. The animals and cultured cells can
be used as in vivo and in vitro systems for the study of the

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role of drug candidates on cell architecture, cell division and apoptosis as well as a morphology of primary cultured neurons, sprouting of neurites, axon branching and axon elongation in primary cultures and also for investigation of organele movement (Fig. 9). They can also be employed as a test system for neurotrophic effects oftested substances.

A study can be designed to screen for a quantitative or qualitative changes in an interaction of tau and tubulin and also in an interaction with organelle trafficking that can be modulated by tested substances.

A study can also be designed to screen for compounds that modulate the expression or activity of the cytoskeletal proteins in the process of building of synaptic connections.

A study can be also designed to screen for the compounds that can affect of NFT formation or protein-protein interaction, which could eventually lead to slowing down, inhibiting or elimination of NFT formation and this could be useful assay for screening for substances with potential of therapeutics for tauopathies and other neurodegenerative disease preferably AD. Effect of the compounds can be evaluated in cell cultures and also in vivo on the animals by means of neuropathological, neurophysiological and behavioural analysis. Many variations of screening methods are known to those of skill in the art and may be applied within the scope of the present invention.

These examples describe particular aspects and embodiments of the invention for illustrative purposes and are not limiting on the scope of the invention as listed in the claims below.

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